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Effect of Diisopropylfluorophosphate on Muscarinic and Gamma-Aminobutyric Acid Receptors in Visual Cortex of Cats (Reprint)

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EFFECT OF DIISOPROPYLFLUOROPHOSPHATE ON MUSCARINIC AND GAMMA-AMINOBUTYRIC ACID RECEPTORS IN VISUAL CORTEX OF CATS

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Summary

Administration of diisopropylfluorophosphate (DFP), an organophosphorus (OP) compound, irreversibly inhibits acetylcholinesterase (AChE) and results in cholinergic hyperactivity. This study investigated muscarinic and gamma-aminobutyric acid (GABA) receptor changes in visual cortex of cats following an acute exposure to DFP. A single acute administration of DFP (4 mg/kg) decreased the number of muscarinic receptors at 2, 10, and 20 hours after treatment. GABA receptors were elevated at 2 and 10 hours but returned to within control levels at 20 hours. No significant alteration in muscarinic or GABA receptor affinity was noted. In all cases cortical AChE activity was inhibited 60-90%. These findings show a down regulation of muscarinic receptors after DFP associated with low AChE activity. GABA receptors also are altered, and may be part of a compensatory mechanism to counteract excess cholinergic stimulation.

One of the primary actions of organophosphorus (OP) compounds is to irreversibly inhibit acetylcholinesterase (AChE), thereby preventing the hydrolysis of acetylcholine. OP's are also known to produce symptoms of acute poisoning, tremor, and convulsions. In addition to these, inhibition of central AChE by organophosphates results in diverse alterations in neurotransmitter systems other than the cholinergic system. We have shown in the cat visual cortex that diisopropylfluorophosphate (DFP), an OP compound, alters levels of dopamine (DA) and 3,4-dihydroxyphenylacetic acid (DOPAC), and increases the DOPAC/DA ratio suggesting increased dopamine turnover (1). We also have evidence of increased gamma-aminobutyric acid (GABA) levels in visual cortex after DFP (2). Others previously have reported alterations in DA systems (3-5) and GABA systems (6,7) after OP exposure in various species. While the specific role

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that noncholinergic systems play during an OP challenge is not known, it is clear the neurochemical changes associated with DFP toxicity are not exclusively cholinergic in nature.

A low spatial frequency loss in the visual evoked response (VER) from cats following DFP (8) which recovers over 10-20 hours, even though AChE activity remains depressed (9) has been reported. This visual recovery without recovery of AChE activity can be reversed by picrotoxin administration (10), suggesting that GABA might be involved. In an effort to better understand the visual recovery process, this study was undertaken to determine if cortical muscarinic and GABA receptors are altered following DFP.

Methods

Animal preparation

Seventeen adult cats (2.5-4.7 kg) were used as subjects in this study. Animals were housed in individual cages and allowed free access to food and water. On the day of the study, cats were anesthetized with halothane in a 3:1 gas mixture of nitrous oxide and carbogen. All areas of surgical incision were infiltrated with lidocaine. The trachea, one femoral artery, and two saphenous veins were cannulated. The animal was paralyzed by intravenous infusion of 30 mg/kg/hr of gallamine triethiodide in an isotonic glucose solution. The animal was ventilated artificially and end tidal CO_2 was maintained near 4%. The cat was held in a stereotaxic head holder and core temperature was maintained near 37°C. Heart rate, blood pressure, lung resistance, and EEG were monitored periodically for analysis of blood gases. Halothane was removed from the gas mixture following completion of all surgical procedures. If any distress was indicated by monitoring of vital signs, halothane was again added to the gas mixture.

Chemicals and Administration of DFP

[^{3}H]QNB (quinuclidinyl benzilate, specific activity, 46.3 Ci/mmol) and [^{3}H]muscimol (specific activity, 20 Ci/mmol) were obtained from New England Nuclear Corporation, Boston, MA. DFP, atropine sulfate, and GABA were obtained from Sigma Chemical Co., St. Louis, MO. American Cyanamid Company provided the gallamine triethiodide. All other reagents and chemicals of analytical grade were obtained from commercial vendors.

Solutions of DFP were prepared in normal saline at a concentration of 10 mg/ml. All treatment animals received a single 4 mg/kg body weight i.v. dose of DFP. Control animals received a saline vehicle injection equivalent to a 4 mg/kg body weight volume. A primary systemic effect of AChE inhibition is stimulated secretory activity. If physiological signs of hypoxia were found following administration of DFP, normality was restored by adjusting ventilation stroke volume and/or aspirating the airway. Following completion of the appropriate survival time, halothane was added once again to the anesthetic gas mixture and samples of visual cortex were removed under deep anesthesia.

Determination of AChE activity

AChE activity was determined according to the method of Ellman et al. (11). Tissue was homogenized in ice cold sodium phosphate buffer (0.1 M, pH 8.0) at a concentration of 20 mg wet weight per ml buffer. Resulting activity is expressed as μmol of acetylthiocholine hydrolyzed/min/mg tissue. Protein was determined by the method of Lowry et al.(12). Bovine serum albumin was used as the standard.

Membrane preparation

Membranes were prepared according to the procedure of Zukin et al. (13) with slight modification. Visual cortex was removed from treatment and control animals under general anesthesia and homogenized by polytron in 15 volumes of ice cold 0.32 M sucrose. The homogenate was centrifuged at 1000 x g for 10 min. The supernatant was collected and centrifuged at 20,000 x g to obtain a pellet. The pellet was resuspended in deionized water and dispersed by polytron. The suspension was centrifuged at 8000 x g for 20 min. The supernatant, including the buffy coat, was collected and centrifuged at 48,000 x g for 20 min. The pellet was suspended in 0.05 M Tris-HCl buffer, pH 7.4, and stored at -80°C until assayed.

[^{3}H]QNB binding

Frozen membranes were thawed and centrifuged at 48,000 x g for 10 min, and the pellet resuspended in 50 mM sodium-potassium buffer (Na-K), pH 7.4. The binding assay was performed in 50 mM Na-K buffer with increasing concentrations of ^{3}H[QNB] (0.01 - 1.0 nM) to a final volume of 1 ml. Specific binding was calculated as the total binding minus that occurring in the presence of 1 μM atropine. Binding was initiated by the addition of prepared membranes (100-200 μg protein) and incubated for 1 hour at 37°C. The reaction was terminated by rapid filtration on GF/B glass fibers. Each filter was washed twice with 5 ml buffer, and the dried filter was transferred to scintillation vials containing 6 ml of PCS II counting solution (Amersham Corporation, Arlington Heights, IL). The radioactivity remaining on the filters was determined by liquid scintillation spectrophotometry.

[^{3}H]muscimol binding

Frozen membranes were thawed and diluted in 10 volumes of ice cold Na-free Tris citrate buffer, 50 mM, pH 7.1. The suspension was centrifuged at 20,000 x g for 10 min and the pellet was resuspended in 50 ml Tris-citrate buffer and incubated at 37°C for 45 min. After incubation, the suspension was recentrifuged at 20,000 x g for 20 min to obtain the final pellet. The binding of [^{3}H]muscimol (0.8-25 nM) was initiated by the addition of membranes (300-400 μg protein) and incubated for 30 min in an ice bath. The reaction was terminated by rapid filtration on GF/B glass fibers. Each filter was washed twice with 5 ml Tris-citrate buffer, and transferred to scintillation vials containing 10 ml of PCS II (Amersham Corporation, Arlington Heights, IL). The radioactivity remaining on the filters was determined by liquid scintillation spectrophotometry. Specific binding was calculated as the total binding minus that occurring in the presence of 100 μM unlabeled GABA.

Statistics

Receptor binding data from the saturation studies were obtained by iterative, nonlinear, least square regression using the radioligand binding analysis program developed by McPherson (14). When applicable, statistical analysis of data was performed by Student's t-test: a p value < 0.05 between two means was considered significant.

Results

TABLE I

Effect of DFP on AChE Activity in Visual Cortex

Treatment	(n)	AChE activity umoles/min/mg tissue	% inhibition from control mean
Control	(4)	4.43 ± .88	-
2 hr post DFP	(3)	1.65 ± .56*	62.7
10 hr post DFP	(2)	1.53 ± .93*	65.4
20 hr post DFP	(3)	.85 ± .07*	80.7

All nonpercent values are mean ± SEM.
*Significantly different from control: p < .05

Effect of DFP on AChE activity

AChE activity in visual cortex following 4.0 mg/kg DFP is shown in Table I. DFP administration produced a significant depression of AChE activity at each time period studied, with the greatest percent inhibition occurring in the 20 hour group.

Effect of DFP on muscarinic receptor binding.

Scatchard analysis of [^{3}H]QNB binding data revealed a single population of muscarinic receptors. DFP (4 mg/kg) significantly decreased the Bmax value by 58% at 2, 46% at 10, and 60% at 20 hours when compared to control values (Fig 1). None of the treatments affected the affinity of the muscarinic receptors (Fig 1).

Effect of DFP on GABA receptor binding.

Scatchard analysis of [^{3}H]muscimol binding data revealed a single high affinity population of GABA receptors. After treatment with DFP (4 mg/kg) the number of GABA receptors in visual cortex, as determined by scatchard analysis, was increased significantly by 130% at 2 hrs and 81% at 10 hrs (Fig 2). Our inability to detect significance from control in the 20-hour treatment group (74% increase) stems from having a large

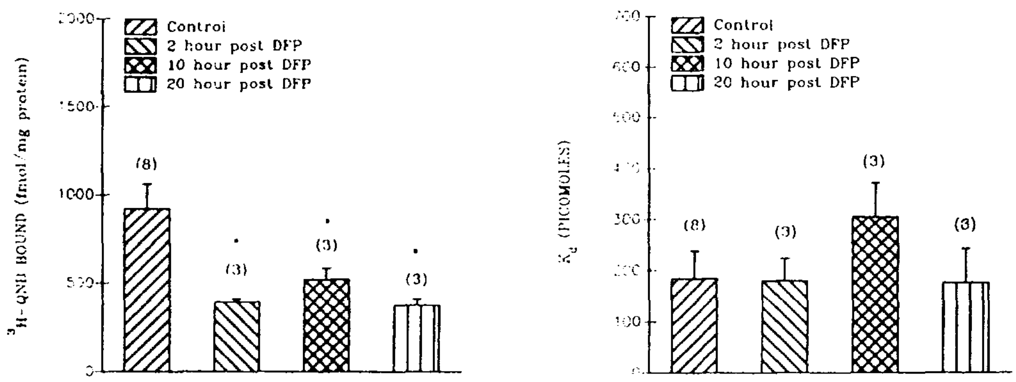

FIG 1.

Effect of DFP on B_{max} and K_d value of ^{3}H-QNB binding in visual cortex of cats. Data in each treatment group are represented as mean ± SEM. Numbers in parentheses represent the size of each treatment group. * indicates $p < 0.05$.

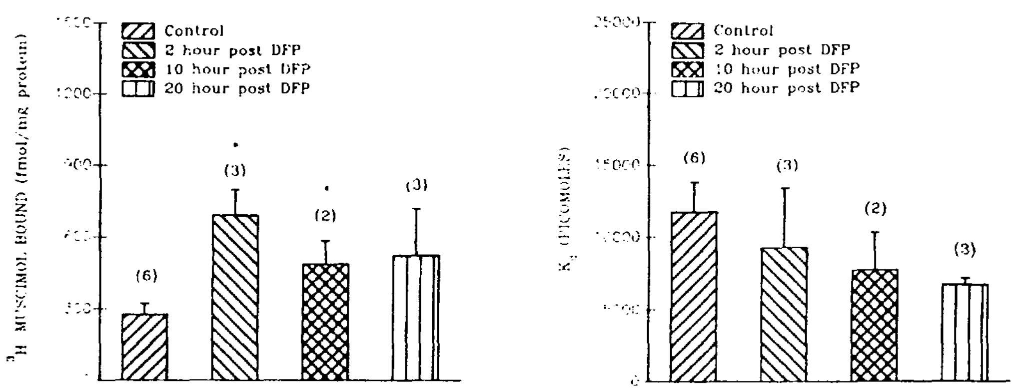

FIG 2.

Effect of DFP on B_{max} and K_d value of ^{3}H-muscimol binding in visual cortex of cats. Data in each treatment group are represented as mean ± SEM. Numbers in parentheses represent the size of each treatment group. * indicates $p < 0.05$.

variability among the observations. No significant change in the affinity of the GABA receptors was noted at any survival time (Fig 2), although there was a downward trend in the data.

Discussion

We found a decrease in muscarinic receptor density in visual cortex of cats after an acute exposure to DFP. No significant change in receptor affinity was noted. The observed decrease in muscarinic receptor density reflects a reaction to excessive cholinergic stimulation initiated by low AChE activity. Little information is available regarding alteration of central muscarinic receptors following DFP in cats, although a down regulation of muscarinic receptors following DFP has been reported previously in rats (15-18). Although it has been fairly well established that muscarinic receptors decrease in number following chronic DFP treatment, such changes are seen following acute treatment in other species only at longer intervals (18,19). We found a decrease in numbers of muscarinic receptors in cat visual cortex at relatively short intervals (2 hrs) following an acute DFP exposure.

We were surprised by the finding shown in Table I that maximum inhibition of AChE in visual cortex occurred 20 hrs after administration of 4.0 mg/kg DFP, rather than in an earlier sample. Both inhibition of blood AChE and maximum reduction of the VER occurs within 2 hrs after a similar dose of DFP (9). This probably indicates that maximum inhibition of cortical AChE occurs during that time as well. Because our experimental animal is the cat, each treatment group is quite small. We are not sure why the maximum cortical inhibition did not appear until 20 hr of survival after DFP, but feel that a larger statistical sample would likely show no difference between the 10- and 20-hour groups. Additionally, this maximum inhibition could be the result of the time period required for equilibration of DFP into the visual cortex.

The number of GABA receptors was increased significantly after DFP. The ubiquity of muscimol binding sites in the visual cortex of the cat suggests that GABA receptors play a critical role in central cortical inhibition (20). The significant increase in GABA receptors at 2 and 10 hrs after DFP and resulting cholinesterase inhibition may occur to reverse the hyperactivity caused by ACh accumulation in the synapse. GABA has been shown to inhibit spontaneous and visually evoked activity of single neurons in the striate cortex of the cat (21). Baclofen, a GABA agonist exerts its effects by interfering specifically with ionic mechanisms at both pre- and postsynaptic sites. In addition, baclofen countered the effects of applying a neuronal excitant (glutamate) and depressed postsynaptic potentials by reducing the release of excitatory amino acids (22). It is suggested that GABAergic neurons act collectively to prevent excitatory neurons from excessive firing. This is supported by studies that show at least in part, the effect of GABA blockade with picrotoxin or bicuculline, resulting in widespread excess neural activity (23,24). Presumably, more GABA receptors make the available GABA more effective in decreasing the excess excitability resulting from low AChE activity. When picrotoxin, a GABA antagonist, is administered after recovery of the visual evoked response following DFP, the recovered VER returns to a post DFP reduced state (10). This provides further support for GABA involvement in the visual recovery process.

We have used the VER as a measure to assess changes in visual response when the cholinergic system is altered. It seems reasonable to conclude that excess cholinergic stimulation mediates the initial reduction of the VER. Harding et al. (25) showed that atropine sulfate reverses the visual loss following physostigmine administration, and subsequent experiments showed a similar reversal following DFP (26). Furthermore, we have shown that the VER can recover over time (18-20 hrs) even though AChE activity remains irreversibly inhibited (9). In retinal release studies (27), DFP exposure (1-2 mM) results in increased release of both DA and GABA, even when Ca^{++} is omitted from the buffer. This suggests that synaptic transmission may not be necessary for increased release. The increase cannot be blocked by the addition of 1 mM atropine or mecamylamine to the buffer solution. If the DFP effect was caused by excess ACh resulting from cholinesterase (ChE) inhibition, 1-2 mM physostigmine or ACh exposure should have given similar results. But there was no increase in GABA or DA release following their addition to the buffer. These studies are consistent with a primary non-cholinergic effect of DFP on retinal neurons and suggest in addition to AChE inhibition that non-specific interactions with other neurotransmitter systems are taking place. This nonspecific interaction could be an alternate explanation to a compensatory mechanism for the VER returning to baseline without need for a concomitant return of the cholinergic system to normalcy. Whether more of the observed effect is due to excessive accumulation of ACh than to secondary factors is not clear. In either case, the exact mechanism of action is still not fully understood.

Finally, we have shown that cortical GABA levels are decreased after DFP in short term survival cats (1-3 hours) and increased in midterm survival animals (6-10 hours). Cortical DA and cortical DOPAC/DA ratios are increased at all time points after DFP. Thus, it appears that DFP initiates a neurochemical imbalance through AChE inhibition, and this may be partially counteracted by immediate increases in dopaminergic activity supported by gradual increases in GABA activity.

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